CLAIMS

WE CLAIM:

1. A polypeptide comprising a Tn5 transposase mutant modified relative to a wild-type Tn5 transposase, the transposase mutant comprising a mutation at position 54, a mutation at position 242, and a mutation at position 372, wherein the transposase mutant has greater avidity than wild-type Tn5 transposase for at least one of a Tn5 outside end sequence as defined by SEQ ID NO:3 and a modified Tn5 outside end sequence as defined by SEQ ID NO:5.

2. A polypeptide as claimed in claim 1 wherein the mutation at position 54 of the Tn5 transposase mutant is a substitution mutation.

3. A polypeptide as claimed in claim 2 wherein position 54 of the Tn5 transposase mutant is a lysine.

4. A polypeptide as claimed in claim 2 wherein position 54 of the Tn5 transposase mutant is a valine.

5. A polypeptide as claimed in claim 1 wherein the mutation at position 372 of the Tn5 transposase mutant is a substitution mutation.

6. A polypeptide as claimed in claim 5 wherein position 372 of the Tn5 transposase mutant is a proline.

7. A polypeptide as claimed in claim 5 wherein position 372 of the Tn5 transposase mutant is a glutamine.

8. A polypeptide as claimed in claim 1 wherein the mutation at position 242 of the Tn5 transposase mutant is a substitution mutation.

9. A polypeptide as claimed in claim 8 wherein position 242 of the Tn5 transposase mutant is an amino acid selected from the group consisting of alanine, glycine, valine, leucine, isoleucine, tyrosine, phenylalanine, and aspartic acid.

10. A polypeptide as claimed in claim 1 wherein the Tn5 transposase mutant further comprises a substitution mutation at position 56, wherein the transposase mutant lacks an inhibitor activity.

11. A polypeptide as claimed in claim 10 wherein position 56 of the Tn5 transposase mutant is an alanine.

12. A Tn5 transposase mutant modified relative to a wild-type Tn5 transposase, the transposase mutant comprising a mutation at position 54, a mutation at position 242, and a mutation at position 372, wherein the transposase mutant has greater avidity than wild-type Tn5 transposase for at least one of a Tn5 outside end sequence as defined by SEQ ID NO:3 and a modified Tn5 outside end sequence as defined by SEQ ID NO:5.

13. A nucleic acid comprising a polynucleotide that encodes the Tn5 transposase mutant as claimed in claim 12.

14. A nucleic acid as claimed in claim 13 further comprising a transcriptional control sequence operably linked to the polynucleotide that encodes the Tn5 transposase mutant.

15. A host cell comprising a nucleic acid as claimed in claim 13.

16. A system for transposing a transposable DNA sequence *in vitro*, the system comprising:

the polypeptide of claim 1;

a donor DNA molecule comprising the transposable DNA sequence, the transposable DNA sequence being flanked at its 5'- and 3'-ends by sequences selected from the group consisting of a wild-type Tn5 outside end sequence and a modified Tn5 outside end sequence that is active for *in vitro* transposition; and

a target DNA molecule into which the transposable DNA sequence can transpose.

17. A method for *in vitro* transposition, the method comprising the steps of:

combining a donor DNA molecule that comprises a transposable DNA sequence of interest with a target DNA molecule and the polypeptide of claim 1 in a suitable reaction buffer for a period of time sufficient for the enzyme to catalyze *in vitro* transposition,

wherein the transposable DNA sequence of interest is flanked at its 5'- and 3'-ends by a pair of outside end sequences selected from the group consisting of a wild-type Tn5 outside end sequence and modified Tn5 outside end sequences that are active for *in vitro* transposition.

18. A method for *in vitro* transposition in a genetic construct that comprises a transposable portion and a donor backbone portion, the transposable portion comprising an origin of replication, a nucleotide sequence of interest, and a pair of outside end sequences flanking the donor backbone portion, the outside end sequences selected from the group consisting of a wild-type Tn5 outside end sequence and modified Tn5 outside end sequences that are active for *in vitro* transposition, the method comprising the steps of:

combining, in an *in vitro* reaction mix, the polypeptide of claim 1 and the genetic construct at a low concentration, to generate reaction products;

transforming the reaction products into a host cell;

proliferating the host cell to generate multiple transformed cells; and

selecting from among the multiple transformed cells for cells that comprise a DNA molecule that has lost the donor backbone portion and that comprise a transposition of the nucleotide sequence of interest.

19. A method for forming a synaptic complex between (a) the polypeptide of claim 1 and (b) a polynucleotide that comprises a pair of outside end sequences and a transposable nucleotide sequence therebetween, wherein the outside sequences are selected from the group consisting of a wild-type Tn 5 outside end sequence and modified Tn5 outside end sequences that are active for *in vitro* transposition, the method comprising the step of:

combining (a) and (b) *in vitro* under conditions that disfavor polynucleotide strand transfer to form the synaptic complex.

20. A method for making an insertional mutation at a random or quasi-random position in cellular nucleic acid in a target cell, the method comprising the step of:

introducing into the target cell a synaptic complex according to claim 19 under conditions that mediate transpositions into the cellular nucleic acid.